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Chemical and Biochemical Properties of the Iron Mineral Core of Mammalian Ferritin.

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19 ABSTRACT (Continue on reverse if necessary and identify by block number)

Both apo and holo ferritin bind Fe^{+} as well as other metal ions (Cu^{2+}, Zn^{2+} and Mn^{2+}) under anaerobic conditions as a function of pH. Apo ferritin binds 8 Fe^{2+} at protein sites whereas holo binds large numbers of Fe^{2+} on its mineral core surface. Holo ferritin undergoes reduction at its FeOOH mineral core forming a Fe^{2+} mineral phase. Apo ferritin also undergoes redox reactions presumable at some amino acid cite. Electron transfer reactions occur readily in the FeOOH core indicating the mineral core has relatively high electrical conductivity.

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ANNUAL REPORT: Contract N00014-88-K-0353

PRINCIPAL INVESTIGATOR: Gerald D. Watt

CONTRACTOR: The University of Colorado

CONTRACT TITLE: Chemical and Biochemical Properties of the Iron Mineral Core of Mammalian Ferritin

START DATE: 6/1/88

RESEARCH OBJECTIVES: To evaluate the chemical composition of the iron core of mammalian ferritin with regard to: 1) binding of metal ions; 2) electrochemical energy storage and 3) electron transfer reactions.

PROGRESS (YEAR 1)

Metal Ion Binding. Holo mammalian ferritin contains up to 4500 iron atoms within its hollow protein interior (80A in diameter) in the form of an FeOOH mineral core. Removal of this iron core by reduction and Fe(II) chelation forms apo ferritin with an empty, hollow interior. We have shown that both apo and holo ferritin avidly bind Fe2+ under anaerobic conditions in a pH dependent binding process. For holo ferritin, the binding occurs at the FeOOH mineral surface while for apo ferritin, Fe2+ binds at 8 protein sites. Cu2+, Zn2+ and Mn2+ also strongly bind to both holo and apo ferritin. These metals are readily removed by chelating agents. Immobilization of both ferritin forms on acrylamide or sepharose supports readily occurs with complete retention of this metal ion binding ability.

Redox Chemistry. The mineral core of mammalian ferritin undergoes complete reduction to a corresponding Fe2+ core (of unknown composition and structure at present). The reduction potential is -310 mv at pH 8 but is strongly pH dependent, suggesting the following overall reaction: $FeOOH + e + 2H^+ = FeOH^+ + H_2O$. The mineral core of ferritin is thus capable of storing large numbers of low potential electrons as well as protons in its isolated interior. Partial reduction of the ferritin core produces discrete Fe(II) and Fe(III) within the core mineral.

Electron Transfer. Addition of labeled $^{57}Fe^{2+}$ to anaerobic holo ferritin produces $^{57}Fe^{3+}$ bound to the mineral core, demonstrating electron transfer to the bulk core and indicating that the mineral core is conductive. Addition of Fe^{2+} specific chelators removes $^{57}Fe^{2+}$, demonstrating the reversibility of this electron transfer reaction. Attempts to determine how redox reagents external to the sequestered mineral core are able to transfer electrons to the interior through the intervening 20-30A protein shell are underway. Three hypotheses are being investigated: 1) Fe^{2+}/Fe^{3+} mediated electron transfer; 2) electron tunneling and 3) protein mediated electron transfer from internal amino acid residues. Evidence for the latter process has been obtained recently.

WORK PLAN (YEAR 2): Having demonstrated the presence of a reduced (Fe^{2+}) core, attempts to reconvert it into the original FeOOH state will be undertaken. This reactivity will demonstrate whether the redox reactions are reversible. We will also establish whether other metal ions (Zn, Cu etc.) are capable of forming aggregated mineral phases within the ferritin interior. Attempts to form magnetite and other iron aggregates will also be undertaken. Finally, we will investigate in more detail the nature of the electron transfer process through the ferritin protein shell.

INVENTIONS: None

PUBLICATIONS:

1. Redox Reactivity of Bacterial and Mammalian Ferritin: Is Reductant Entry into the Ferritin Interior a Necessary Step for Iron Release?

   Proc. Natl. Acad. Sci. USA. (1989) 85, 7457-7461.

2. Redox Reactions Associated with Iron Release from Mammalian Ferritin.

   Biochemistry (1989) 28,1650-1655.

3. Fe^{2+} Binding to Apo and Holo Mammalian Ferritin

   In Press, **Biochemistry**

TRAINING ACTIVITIES: The following undergraduate research students (all US citizens) were supported during the past year: Steve Hilty (Occidental, College); Chris Van Buren (University of Colorado) and Chi Hua Chiu (University of Colorado).